## R E M A R K S

Claims 1-3 and 5-14 are pending in this application. Claims 1, 3 and 5-14 are rejected and claim 2 is withdrawn from consideration. No amendments have been made by way of the present submission, thus, no new matter has been added.

In view of the following remarks, Applicants respectfully request that the Examiner withdraw all rejections and allow the currently pending claims.

**Pending Issues under 35 U.S.C. 103(a)**

The Examiner has rejected claims 1, 3, 5-9 and 12-14 under 35 U.S.C. 103(a) as being obvious over Padegimas et al. (Analytical Biochem., Vol. 260, pages 149-153, 1998) in view of Deugau et al. (U.S. Patent 5,858,656). Applicants respectfully traverse this rejection.

The present invention relates to a process for preparing a library of DNA fragments, of which terminal sequences are known, by using a DNA having a base sequence, which is completely unidentified. According to the present invention, an adaptor that is utilized has a single-strand cohesive end group, not a double-strand. The present adaptor is designed to have a hairpin loop structure comprising a complementary and non-complementary region, which structure is formed by intramolecular hybridization. The adaptor is ligated with the digested fragments complementarily.

The common base sequence within the adaptor is used as the primer for PCR amplification and the hairpin loop structure resists exonuclease treatment without chemical modification, which avoids non-specific amplification. Considering the primary reference of Padegimas, several distinctions exist.

Padegimas does not perform a cohesive ("sticky end") ligation. In the present claims, the base DNA is digested into fragments utilizing restriction enzymes. These fragments have single-strand cohesive ends as mentioned above. These ends are then ligated with hairpin loop adaptors having complementary single-strand cohesive ends. In contrast, according to Padegimas, prior to such ligation, the digested genomic DNA is treated with the Klenow fragment of *E.coli* DNA polymerase 1 along with dNTPs (referred to at page 150, second full paragraph, left hand column of Padegimas). The Klenow fragment has both polymerase and 3'-5' exonuclease activity. This step of Padegimas serves to either "fill-in" 5' overhangs resulting from the restriction, or remove 3' overhangs due to the 3'-5' exonuclease activity. Thus, although Padegimas may originally create DNA fragments having single-strand cohesive ends, these cohesive ends are removed due to the Klenow fragment treatment. This deficiency cannot be cured by the secondary reference, Thus no *prima facie* case of obviousness exists.

Also, as indicated by the Examiner, Padegimas fails to disclose the step of eliminating the hairpin loop structure from

the DNA fragments which contain hairpin loop adaptors. Regardless, the Examiner asserts that it would have been obvious to eliminate these hairpin loop structures utilizing the secondary reference of Deugau. Applicants disagree and submit that even if the references of Padegimas and Deugau were combined, the presently claimed subject matter would not result. This is explained below.

Padegimas relates to isolation of an unknown sequence flanking a known DNA sequence. The adaptor contains a longer strand and a shorter strand. The 5′ end of the shorter adaptor strand is phosphorylated to enable ligation with genomic DNA, so that the adaptor resists the 3′-5′ proofreading activity of DNA polymerase and cannot be digested with exonuclease III. Although Padegimas may originally create DNA fragments having single-strand cohesive ends, these cohesive ends are removed due to the Klenow fragment treatment prior to ligation with a hairpin loop adaptor. Also, the oligonucleotides cannot be ligated complementarily with the longer strand due to phosphorothioate linkage at the 3′ end of the shorter strand adaptor.

Deugau discloses only a double stranded adaptor made from the hybridization of the two independent single stranded oligonucleotides. However, Deaugau failts to suggest or disclose a hairpin loop adaptor. Deugau discloses only that the

DNA fragments and adaptors, which did not participate in the ligation reaction, were removed by a washing process.

Accordingly, it can be seen that several distinctions exist between the present invention and the cited art. Contrary to the Examiner's assertion, Padegimas fails to suggest or disclose the currently claimed subject matter. These deficiencies cannot be cured by the secondary reference of Deugau. Accordingly, the Examiner has failed to present a valid *prima facie* case of obviousness. Reconsideration and withdrawal of this rejection are respectfully requested.

The Examiner has also rejected claims 10 and 11 under 35 U.S.C.103(a) as being obvious over Padegimas et al. (Analytical Biochem., Vol. 260, pages 149-153, 1998) in view of Backman et al. (U.S. Patent 5,516,663).

Applicants respectfully traverse this rejection. As discussed above, several differences exist in the primary reference of Padegimas. These deficiencies, similar to Deugau, cannot be cured by the Backman reference. Thus, for the above reasons alone, the Examiner's rejection fails.

Applicants point out that the claimed invention is directed to open only the hairpin loop to increase the efficiency of annealing the primer to the sequence within the adaptor during PCR. In contrast, Padegimas fails to suggest or disclose the step of eliminating the hairpin loop structure from the DNA

fragments, which contain the hairpin loop adaptors. Backman attach a blocking group at the 3' end of the upstream probe to reduce the background noise and contamination during the reaction. Further, endonuclease IV digests the blocking group of the probe only adhering specifically to the template. Accordingly, the Examiner has failed to present a valid *prima facie* case of obviousness. Reconsideration and withdrawal of the outstanding rejection are respectfully requested.

In view of the above, Applicants respectfully submit that each of the Examiner's outstanding rejections are improper.

The Examiner is therefore respectfully requested to withdraw all rejections and allow the currently pending claims.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Craig A. McRobbie (Reg. No. 42,874) at the telephone number of the undersigned below.

Pursuant to 37 C.F.R. §§ 1.17 and 1.136(a), Applicant(s) respectfully petition(s) for a three (3) month extension of time for filing a reply in connection with the present application, and the required fee of $950.00 is attached hereto.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

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